

2.6. Small-angle techniques

BY O. GLATTER AND R. MAY

2.6.1. X-ray techniques (By O. Glatter)

2.6.1.1. Introduction

The purpose of this section is to introduce small-angle scattering as a method for investigation of nonperiodic systems. It should create an understanding of the crucial points of this method, especially by showing the differences from wide-angle diffraction. The most important concepts will be explained. This article also contains a collection of the most important equations and methods for standard applications. For details and special applications, one must refer to the original literature or to textbooks; the reference list is extensive but, of course, not complete.

The physical principles of scattering are the same for wide-angle diffraction and small-angle X-ray scattering. The electric field of the incoming wave induces dipole oscillations in the atoms. The energy of X-rays is so high that all electrons are excited. The accelerated charges generate secondary waves that add at large distances (far-field approach) to give the overall scattering amplitude. All secondary waves have the same frequency but may have different phases caused by the different path lengths. Owing to the high frequency, it is only possible to detect the scattering intensity – the square of the scattering amplitude – and its dependence on the scattering angle.

The angle-dependent scattering amplitude is related to the electron-density distribution of the scatterer by a Fourier transformation. All this holds for both wide-angle diffraction and small-angle X-ray scattering. The main difference is that in the former we have a periodic arrangement of identical scattering centres (particles), *i.e.* the scattering medium is periodic in all three dimensions with a large number of repetitions, whereas in small-angle scattering these particles, for example proteins, are not ordered periodically. They are embedded with arbitrary orientation and with irregular distances in a matrix, such as water. The scattering centres are limited in size, non-oriented, and nonperiodic, but the number of particles is high and they can be assumed to be identical, as in crystallography.

The Fourier transform of a periodic structure in crystallography (crystal diffraction) corresponds to a Fourier series, *i.e.* a periodic structure is expanded in a periodic function system.

The Fourier transform of a non-periodic limited structure (small-angle scattering) corresponds to a Fourier integral. In mathematical terms, it is the expansion of a nonperiodic function by a periodic function system.

So the differences between crystallography and small-angle scattering are equivalent to the differences between a Fourier series and a Fourier integral. It may seem foolish to expand a non-periodic function with a periodic function system, but this is how scattering works and we do not have any other powerful physical process to study these structures.

The essential effect of these differences is that in small-angle scattering we measure a continuous angle-dependent scattering intensity at discrete points instead of sharp, point-like spots as in crystallography.

Another important point is that in small-angle scattering we have a linear increase of the signal (scattered intensity) with the number of particles in the measuring volume since intensities are adding. Amplitudes are adding in crystallography, so we have a quadratic relation between the signal and the number of particles.

In addition, there is a loss of information in small-angle scattering experiments caused by the averaging over all orientations in space. The three-dimensional structure is represented by a one-dimensional function – the dependence of the scattered intensity on the scattering angle. This is also true for powder diffraction. To recover the structure uniquely is therefore impossible.

The computation of the scattering function for a known structure is called the solution of the scattering problem. This problem can be solved exactly for many different structures. The inversion, *i.e.* the estimation of the structure of the scatterer from its scattering functions, is called the inverse scattering problem. This problem cannot be solved uniquely.

The description and solution of the scattering problem gives information to the experimenter concerning the scattering functions to be expected in a special situation. In addition, this knowledge is the starting point for the evaluation and interpretation of experimental data (solution of the inverse problem).

There are methods that give a rough first-order approximation to the solution of the inverse scattering problem using only a minimum amount of *a priori* information about the system to obtain an initial model. In order to improve this model, one has to solve the scattering problem. The resulting theoretical model functions are compared with the experimental data. If necessary, model modifications are deduced from the deviations. After some iterations, one obtains the final model.

It should be noticed that it is possible to find different models that fit the data within their statistical accuracy. In order to reduce this ambiguity, it is necessary to have additional independent information from other experiments. Incorrect models, however, can be rejected when their scattering functions differ significantly from the experimental data.

What type of investigations can be performed with small-angle scattering? It is possible to study monodisperse and polydisperse systems. In the case of monodisperse systems, it is possible to determine size, shape, and, under certain conditions, the internal structure. Monodispersity cannot be deduced from small-angle scattering data and must therefore be assumed or checked by independent methods.

For polydisperse systems, a size distribution can be evaluated under the assumption of a certain shape for the particles (*particle sizing*).

All these statements are strictly true for highly diluted systems where the interparticle distances are much larger than the particle dimensions. In the case of semi-dilute systems, the result of a small-angle scattering experiment is influenced by the structure of the particles and by their spatial arrangement. Then the scattering curve is the product of the particle scattering function and of the interparticle interference function. If the scattering function of one particle is known, it is possible to evaluate information about the radial distribution of these particles relative to each other. If the system is dense, *i.e.* if the volume fraction of the particles (scattering centres) is of the same order of magnitude as the volume fraction of the matrix, it is possible to determine these volume fractions and a characteristic length of the phases. The most important practical applications, however, pertain to dilute systems.

How are small-angle scattering experiments related to other scattering experiments? Small-angle scattering uses radiation with a wavelength in the range 10^{-1} to 10^0 nm, depending on the

2. DIFFRACTION GEOMETRY AND ITS PRACTICAL REALIZATION

problem and on the source used. This range is similar for X-rays and neutrons, but neutrons interact with the nuclei of the atoms whereas X-rays interact with the electrons. The scattering efficiency increases linearly with the atomic number for X-rays. The dependence is much more complicated for neutrons and does not show a systematic trend. The essential fact in neutron scattering is the pronounced difference in the scattering power between hydrogen and deuterium, which is important for varying the contrast between the particles and the matrix.

The wavelength and the scattering efficiency limit the range of small-angle scattering experiments to systems in the size range of a few nanometres up to about 100 nm. Special instruments permit the study of larger particles (Bonse & Hart, 1967; Koch, 1988). These instruments need a high intensity of the primary beam (synchrotron radiation) and are not very common.

Particles in the size range from 100 nm up to some micrometres can be investigated by static light-scattering techniques (Glatter, Hofer, Jorde & Eigner, 1985; Glatter & Hofer, 1988*a,b*; Hofer, Schurz & Glatter, 1989). Particles exceeding this limit can be seen in an optical microscope or can be studied with Fraunhofer diffraction (Bayvel & Jones, 1981).

Electron microscopy is in competition with all these scattering methods. It has the marked advantage of giving real pictures with rather high resolution but it has the inherent disadvantage that the preparation may introduce artefacts. Small-angle scattering, on the other hand, is a method to study macromolecules in solution, which is a very important advantage for biological samples and for polymers.

Crystallography gives more information about the particle (atomic structure) and can be applied to relatively large systems. It is possible to study particles as large as proteins and viruses if good crystals of these substances are available. The experiment needs synchrotron radiation for large molecules like proteins or viruses, *i.e.* access to a large research facility is necessary. Small-angle X-ray scattering with conventional generators is a typical next-door technique with the advantage of ready availability.

Small-angle scattering has developed into a standard measuring method during recent decades, being most powerful for the investigation of submicrometre particles.

2.6.1.2. General principles

In this subsection, we are concerned with X-rays only, but all equations may also be applied with slight modifications to neutron or electron diffraction. When a wave of X-rays strikes an object, every electron becomes the source of a scattered wave. All these waves have the same intensity given by the Thomson formula

$$I_e(\theta) = I_p T_f \frac{1 + \cos^2 2\theta}{2}, \quad (2.6.1.1)$$

where I_p is the primary intensity and a the distance from the object to the detector. The factor T_f is the square of the classical electron radius ($e^2/mc^2 = 7.90 \times 10^{-26}[\text{cm}^2]$). The scattering angle 2θ is the angle between the primary beam and the scattered beam. The last term in (2.6.1.1) is the polarization factor and is practically equal to 1 for all problems dealt with in this subsection. I_e should appear in all following equations but will be omitted, *i.e.* the amplitude of the wave scattered by an electron will be taken to be of magnitude 1. I_e is only needed in cases where the absolute intensity is of interest.

The amplitudes differ only by their phases φ , which depend on the positions of the electrons in space. Incoherent (Compton) scattering can be neglected for small-angle X-ray scattering. The

phase φ is $2\pi/\lambda$ times the difference between the optical path length of the wave and an arbitrary reference wave (with λ being the wavelength). The direction of the incident beam is defined by the unit vector \mathbf{s}_0 and of the scattered beam by \mathbf{s} . The angle between these two unit vectors (scattering angle) is 2θ . The path difference between the rays through a point P and an arbitrary origin O is $-\mathbf{r}(\mathbf{s} - \mathbf{s}_0)$. The phase is $\varphi = -\mathbf{h} \cdot \mathbf{r}$ if we define the scattering vector \mathbf{h} as

$$\mathbf{h} = (2\pi/\lambda)(\mathbf{s} - \mathbf{s}_0). \quad (2.6.1.2)$$

This vector bisects the angle between the scattered beam and the incident beam and has length $h = (4\pi/\lambda) \sin \theta$. We keep in mind that $\sin \theta$ may be replaced by θ in small-angle scattering. We now introduce the electron density $\rho(\mathbf{r})$. This is the number of electrons per unit volume at the position \mathbf{r} . A volume element dV at \mathbf{r} contains $\rho(\mathbf{r}) dV$ electrons. The scattering amplitude of the whole irradiated volume V is given by

$$A(\mathbf{h}) = \iiint \rho(\mathbf{r}) \exp(-i\mathbf{h} \cdot \mathbf{r}) dV. \quad (2.6.1.3)$$

We see that the amplitude A is the Fourier transform of the electron-density distribution ρ . The intensity $I(\mathbf{h})$ of the complex amplitude $A(\mathbf{h})$ is the absolute square given by the product of the amplitude and its complex conjugate A^* ,

$$I(\mathbf{h}) = A(\mathbf{h})A(\mathbf{h})^* = \iiint \tilde{\rho}^2(\mathbf{r}) \exp(-i\mathbf{h} \cdot \mathbf{r}) dV, \quad (2.6.1.4)$$

where $\tilde{\rho}^2(\mathbf{r})$ is the convolution square (Bracewell, 1986):

$$\tilde{\rho}^2(\mathbf{r}) = \iiint \rho(\mathbf{r}_1)\rho(\mathbf{r}_1 - \mathbf{r}) dV_1. \quad (2.6.1.5)$$

The intensity distribution in \mathbf{h} or reciprocal space is uniquely determined by the structure in real space.

Until now, we have discussed the scattering process of a particle in fixed orientation in vacuum. In most cases of small-angle scattering, the following situation is present:

-The scatterers (particles or inhomogeneities) are statistically isotropic and no long-range order exists, *i.e.* there is no correlation between points at great spatial distance.

-The scatterers are embedded in a matrix. The matrix is considered to be a homogeneous medium with the electron density ρ_0 . This situation holds for particles in solution or for inhomogeneities in a solid. The electron density in equations (2.6.1.3)–(2.6.1.5) should be replaced by the difference in electron density $\Delta\rho = \rho - \rho_0$, which can take positive and negative values.

The average over all orientations $\langle \rangle$ leads to

$$\langle \exp(-i\mathbf{h} \cdot \mathbf{r}) \rangle = \frac{\sin hr}{hr} \quad (2.6.1.6)$$

(Debye, 1915) and (2.6.1.4) reduces to the form

$$I(h) = 4\pi \int_0^\infty r^2 \Delta\tilde{\rho}^2(r) \frac{\sin hr}{hr} dr \quad (2.6.1.7)$$

or, with

$$p(r) = r^2 \Delta\tilde{\rho}^2(r) = r^2 V \gamma(r), \quad (2.6.1.8)$$

to

$$I(h) = 4\pi \int_0^\infty p(r) \frac{\sin hr}{hr} dr; \quad (2.6.1.9)$$

γ is the so-called correlation function (Debye & Bueche, 1949), or characteristic function (Porod, 1951). The function $p(r)$ is the so-called pair-distance distribution function PDDF (Guinier &